Double stranded DNA sequencing (1) is favored because of its simplicity, and convenience. However it is only recently that the quality of this method has become comparable with single stranded DNA sequencing. We believe that two factors have limited the popularity of double stranded DNA sequencing: 1. the quality of the template DNA; 2. the inherent properties of the DNA polymerase. The modified T7 DNA polymerase (Sequenase™) has several properties which make it more suitable for sequencing (2). We replaced the Klenow Polymerase I with Sequenase™ in our double stranded DNA sequencing, and by paying careful attention to the conditions used to denature and recover the template DNA, we are now routinely producing sequencing results which are as good as single stranded DNA sequencing (see figure). Here, we present a step-by-step protocol for the alkaline denaturation of template DNA, and our recommendations for the sequencing reaction.

I. Template preparation:
1. use 3μg of CsCl purified plasmid DNA, add NaOH to 0.2M and EDTA to 0.2mM (total volume 20μl), incubate at room temperature for 5min
2. neutralize by adding 2μl of 2M NH₄Ac (pH4.6), mix quickly, then add 60μl of 100% ethanol (-20°C), mix well on ice
3. precipitate the DNA in a microfuge for 20min at 4°C, wash once with 80% ethanol (-20°C), spin again for another 5min
4. carefully draw away ethanol, and dry the DNA in a vacuum desicator for 10min, then use immediately

II. Sequencing Reaction:
See Sequenase™ Manual (3) for details. This manual was originally designed for single stranded DNA sequencing, for double stranded DNA sequencing, we recommend the following modifications:
1. 5ng (about 1pmol) primer will be enough, excess of primer often decreases the sequencing quality
2. anneal template and primer at 65°C for 3-5min, then cool at room temperature for about 30min
3. 1ul of 400 Ci/mmol of (35S)-dATP per reaction will give satisfactory results

References:
3. Step-By-Step Protocols For DNA Sequencing With Sequenase™ (United States Biochemical Corporation, P.O.Box 22400, Cleveland, Ohio 44122)